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### Permalink

<https://escholarship.org/uc/item/6pc771sg>

### Journal

Journal of clinical biochemistry and nutrition, 56(2)

### ISSN

0912-0009

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### Publication Date

2015-03-01

### DOI

10.3164/jcbn.14-111

Peer reviewed

# Combined treatment with dipeptidyl peptidase 4 (DPP4) inhibitor sitagliptin and elemental diets reduced indomethacin-induced intestinal injury in rats via the increase of mucosal glucagon-like peptide-2 concentration

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(Received 3 September, 2014; Accepted 6 October, 2014; Published online 28 January, 2015)

The gut incretin glucagon-like peptide-1 (GLP-1) and the intestinotropic hormone GLP-2 are released from enteroendocrine L cells in response to ingested nutrients. Treatment with an exogenous GLP-2 analogue increases intestinal villous mass and prevents intestinal injury. Since GLP-2 is rapidly degraded by dipeptidyl peptidase 4 (DPP4), DPP4 inhibition may be an effective treatment for intestinal ulcers. We measured mRNA expression and DPP enzymatic activity in intestinal segments. Mucosal DPP activity and GLP concentrations were measured after administration of the DPP4 inhibitor sitagliptin (STG). Small intestinal ulcers were induced by indomethacin (IM) injection. STG was given before IM treatment, or orally administered after IM treatment with or without an elemental diet (ED). DPP4 mRNA expression and enzymatic activity were high in the jejunum and ileum. STG dose-dependently suppressed ileal mucosal enzyme activity. Treatment with STG prior to IM reduced small intestinal ulcer scores. Combined treatment with STG and ED accelerated intestinal ulcer healing, accompanied by increased mucosal GLP-2 concentrations. The reduction of ulcers by ED and STG was reversed by co-administration of the GLP-2 receptor antagonist. DPP4 inhibition combined with luminal nutrients, which up-regulate mucosal concentrations of GLP-2, may be an effective therapy for the treatment of small intestinal ulcers.

**Key Words:** GLP-1, GLP-2, DPP4, DPP8, sitagliptin

The gut incretin hormones, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide/glucose-dependent insulinotropic peptide (GIP), are released from enteroendocrine cells in response to ingested nutrients and augment insulin secretion.<sup>(1)</sup> Although GLP-1 like peptides are used therapeutically to treat type 2 diabetes, native GLP-1 is rapidly degraded and deactivated by dipeptidyl peptidase-4 (DPP4).<sup>(2)</sup> Gliptin inhibitors of DPP4 activity such as sitagliptin (STG), vildagliptin, and saxagliptin, work by slowing incretin metabolism, increasing endogenous GLP-1 concentrations, and improving post-prandial glycemic control in type 2 diabetes.<sup>(3,4)</sup>

Another processing product of proglucagon, GLP-2 is also a DPP4 substrate. GLP-2 is a pleiotropic hormone that affects

multiple facets of intestinal physiology, including epithelial growth, barrier function, digestion, absorption, motility, and blood flow.<sup>(5,6)</sup> Since GLP-2 exerts potent trophic and anti-apoptotic effects in the gastrointestinal tract and improves intestinal absorption, GLP-2 is an attractive target for development of novel treatments for intestinal diseases.<sup>(7,8)</sup> The stable GLP-2 analogue teduglutide significantly increases intestinal wet weight and improves nutrient absorption in short bowel syndrome patients,<sup>(9)</sup> although teduglutide therapy is somewhat risky and quite costly. Since GLP-2 is also rapidly degraded and deactivated by DPP4, DPP4 inhibition has also been considered as a novel approach for the treatment of inflammatory bowel disease based on experimental and clinical studies.<sup>(10-13)</sup> However, the effects of DPP4 inhibition on other forms of intestinal damage such as ulcers due to non-steroidal anti-inflammatory drugs (NSAIDs) have not been extensively explored. Although GLP-2 induces its intestinotrophic effects through endocrine and paracrine mechanisms, the potential mucosal protective effect of local GLP-2 has not been explored.<sup>(5,14)</sup> Furthermore, the effects of DPP4 inhibitors on intestinal GLP concentrations and DPP4 activity have not been studied.

NSAIDs such as low-dose aspirin are widely used to reduce the risk of cardio- and cerebrovascular thrombosis.<sup>(15,16)</sup> However, NSAIDs often induces gastroenteropathy by increasing the permeability of mucosa.<sup>(17,18)</sup> So far, various drugs such as misoprostol, sulfasalazine, anti-biotics, and polaprezinc has been considered to be candidate drugs to NSAID-induced intestinal injury.<sup>(19)</sup> And it is reported that combined treatment with rebamipid and proton pump inhibitor omeprazole prevented NSAID-induced gastrointestinal symptoms, especially lower gastrointestinal symptoms.<sup>(20)</sup> Although potent anti-secretory therapy can prevent NSAID-induced foregut mucosal injury, the usefulness of proton pump inhibitor is still controversial.<sup>(21)</sup>

We recently reported that exogenous GLP-2 or a DPP4 inhibitor prevented ulcer formation and promoted healing of NSAID-induced intestinal ulcers in rats.<sup>(22)</sup> Furthermore, DPP4 inhibition with amino acids and a nucleotide umami taste receptor (TAS1R3)

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ligand accelerated intestinal ulcer healing, suggesting that DPP4 inhibition combined with luminal amino acids enhances GLP-2-related mucosal protection, thus preventing ulceration and promoting healing of NSAID-induced enteropathy.

Therefore, we further hypothesized that the clinically used DPP4 inhibitor STG prevents intestinal ulcers and promotes ulcer healing by inhibiting mucosal DPP4, which increases mucosal GLP-2 concentrations. We also examined the effect of an elemental diet (ED), which is used therapeutically for Crohn's disease patients,<sup>(23,24)</sup> on healing of NSAID-induced intestinal ulcers.

## Materials and Methods

**Animals.** Six-week-old male BALB/c mice (CLEA Japan, Tokyo, Japan) weighing 20–25 g and six-week-old male SD rats (Oriental Bio Service, Inc., Kyoto, Japan) weighing 200–250 g were used in this study. The animals were maintained in an animal colony with controlled temperature (23°C) and light (12 h light–dark cycle) at the Osaka Medical College, Osaka, Japan, and were permitted free access to standard mouse or rat chow pellets (MM-3, Funabashi Farms, Chiba, Japan) and tap water. Ethical permission for the experiments presented in this study was given by the Animal Ethical Committee of the Osaka Medical College and all procedures were conducted according to the guidelines of the Institute for Laboratory Animal Research at Osaka Medical College.

**Chemicals and elemental diets.** The DPP4 inhibitor STG phosphate monohydrate was purchased from Carbosynth Limited (Berkshire, UK). STG phosphate monohydrate was dissolved in distilled water and given to mice by oral gavage at a dose of 0.3, 1, or 3 mg/kg. Another DPP4 inhibitor, K-579, was obtained from Tocris Bioscience (Ellisville, MO). K-579 was dissolved in dimethyl sulfoxide at a concentration of 0.1 M to make a stock solution. GLP-2<sub>3–33</sub> was synthesized by Operon Biotechnologies, K.K. (Tokyo, Japan). Elental® (Ajinomoto Co., Inc., Tokyo, Japan) was used as the elemental diet, and contains essential amino acids as the sole source of nitrogen. Indomethacin (IM) and other chemicals were obtained from Sigma Chemical (St. Louis, MO). IM was freshly dissolved in ethanol.

### Analysis of DPP4 mRNA expression by real-time PCR.

To evaluate segmental differences in mucosal DPP4 mRNA expression in mouse intestine, scraped mucosal samples were removed from the duodenum, jejunum, ileum, proximal colon (PC), and distal colon (DC), frozen in liquid nitrogen, and stored at –80°C until RNA isolation. Total RNA was extracted using an RNeasy mini-Kit (Qiagen in Japan, Tokyo, Japan). RNA was reverse-transcribed with a Prime Script RT reagent Kit (Takara Bio Inc., Shiga, Japan) with a SYBR Premix Ex Taq Kit (Takara) using a Thermal Cycler Dice Real Time System TP870 (Takara). Sequences of the sense and antisense primers for mouse DPPs and  $\beta$ -actin were as follows: DPP4: 5'-AAATGGGATTTGTGGACAGCAAG-3' and 5'-CCGATCCCAGGACCATTGAG-3'; DPP8: 5'-GCGGTAAGCTGATCCCTGACA-3' and 5'-CCAGCCAGC-ACTTGAAAGCTC-3'; DPP9: 5'-TCATCCACAAGCCACAAGTGTTTC-3' and 5'-GGGCTACAGACCCTGCCTCATA-3';  $\beta$ -actin: 5'-CATCCGTAAAGACCTCTATGCCAAC-3' and 5'-ATGGAGCCACCGATCCACA-3'. Cycling conditions were as follows: 1 cycle of 95°C for 30 s for denaturing followed by 40 cycles of 95°C for 5 s; 60°C for 30 s, 1 cycle of 95°C for 15 s, and 60°C for 30 s. The expression of target genes was normalized to expression of  $\beta$ -actin.

**Mucosal DPP activity.** Mice were assigned to groups receiving either sitagliptin (0.3, 1, or 3 mg/kg) or 0.2 ml of saline vehicle via oral gavage. Mice were sacrificed 24 h after STG administration. Mice were freely fed until euthanasia. Mucosal scrapes of each intestinal segment were collected in homogenization buffer (Tris-buffered saline) containing protease inhibitors (Complete Mini, Roche Diagnostics, Indianapolis, IN). Blood samples were collected into chilled Eppendorf tubes containing

1  $\mu$ l of 0.5 M EDTA. The homogenate and blood samples were immediately centrifuged at 3,000  $\times$  g for 5 min, and supernatants or plasma were stored at –80°C until measurements were taken. Mucosal DPP activity was determined by the cleavage rate of 7-amino-4-methylcoumarin (Sensolyte AMC DPP4 Assay Kit, AnaSpec, Inc., Fremont, CA) from the synthetic substrate; this assay was performed according to the manufacturer's instructions. In brief, mucosal lysate containing 10  $\mu$ g/50  $\mu$ l of protein was mixed with 50  $\mu$ l of DPP substrate AMC (AnaSpec). After 30 min of incubation at room temperature, cleaved AMC was measured using a fluorescence plate reader with excitation at 354 nm and emission at 442 nm (Fluoro Skan Ascent, Thermo Fisher Scientific K.K., Kanagawa, Japan). Mucosal DPP activity was expressed as relative fluorescence units (RFU).

**Measurement of plasma and mucosal GLPs.** Plasma and mucosal homogenates were processed in the presence of K-579 (final concentration 0.1 mM) as described above. Plasma and mucosal concentrations of active GLP-1<sub>7–36</sub> and total GLP-2 were measured using a GLP-1 (Active 7–36) ELISA kit and a GLP-2 (Mouse) ELISA kit (ALPCO Diagnostics, Salem, NH) according to the manufacturer's instructions.

**Immunohistochemistry and immunoblot analyses.** Expression of single-stranded DNA (ssDNA) in the intestinal mucosa was assessed by the labeled streptavidin-biotin method using an LSAB kit (Dako Japan, Tokyo, Japan) with microwave antigen retrieval. The ileum was fixed in 10% formalin, paraffin-embedded, and cut into 4- $\mu$ m tissue sections. The sections were mounted on microscope slides, deparaffinized in xylene, and dehydrated with 100% ethanol. After washing with PBS, sections were placed in 10 mM citrate buffer (pH 6.0) and heated to 80°C for 10 min in a microwave oven. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide in 10% methanol for 30 min, followed by incubation with blocking reagent for 15 min. Sections were incubated at 4°C overnight with rabbit anti-ssDNA antibody (Dako). After washing with PBS, sections were incubated with biotinylated anti-rabbit IgG (Dako) at room temperature for 30 min. Sections were then visualized using streptavidin-biotin horseradish peroxidase (Dako) and 3,3' diaminobenzidine (Dako). Sections were counterstained with hematoxylin, dehydrated, and cover-slipped with permanent mounting medium for microscopic observation.

Lysates of ileal mucosa containing 20  $\mu$ g/20  $\mu$ l of protein were separated by electrophoresis through a NuPAGE in 4–12% Bis-Tris Gel 1.0 mm (Life Technologies, California, USA), and transferred onto polyvinylidene difluoride membranes (Pall Corporation, New York, NY). Membranes were incubated in 5% skim milk in Tris-buffered saline with Tween 20 (TBST) at room temperature for 10 min and stored at 4°C overnight. After washing 3 times in TBST, membranes were incubated with rabbit anti-cleaved caspase-3 antibody (Cell Signaling Technology Japan, Tokyo, Japan) and mouse anti- $\beta$ -actin antibody (Sigma) for 1 h at room temperature in 5% skim milk. Membranes were washed 3 times in TBST and incubated with peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Dallas, TX). Blots were washed 3 times with TBST and developed using an enhanced chemiluminescence system (ECL Prime Western Blotting Detection System, Buckinghamshire, UK, and FUJIFILM Imaging System Application Note LAS-3000, FUJIFILM, Tokyo, Japan).

### Effects of STG on indomethacin-induced intestinal injury.

For the ulcer prevention study, small intestinal ulcers were induced by IM treatment (10 mg/kg/day, s.c.) in fed rats on Day 0 as previously described.<sup>(22)</sup> The DPP4 inhibitor STG (1 or 3 mg/kg) was given orally for 2 days at 24 h and 0 h before IM treatment. Rats were sacrificed 24 h after IM treatment. The small intestine was divided into 7 segments from duodenum to ileum, and longitudinal ulcer length was measured in each segment as previously described.<sup>(22)</sup> Total ulcer length was expressed as total ulcer score. To identify the effects of endogenous active GLP-2,

GLP-2<sub>3-33</sub> (100 µg/kg/day, s.c.) was administered to STG-treated rats on Day -1 and Day 0 before IM treatment.

For the ulcer healing study, small intestinal ulceration was induced by IM treatment (8 mg/kg/day, s.c. for 2 days) in fed rats as reported previously.<sup>(8)</sup> STG (3 mg/kg/day) was administered orally from Day 2 to Day 8 after IM treatment. For ED treatment, free access to powdered ED instead of standard chow diet was provided daily. Vehicle-treated control animals were given powdered standard chow diet. Rats were euthanized 7 days after IM treatment (Day 8). To identify the effects of endogenous active GLP-2, GLP-2<sub>3-33</sub> (100 µg/kg/day, s.c.) was administered to rats co-treated with STG and ED from Day 2 to Day 8 after IM treatment.

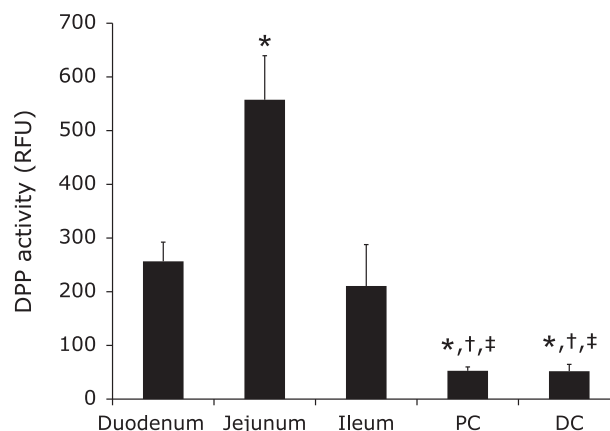
**Statistics.** All data are expressed as means ± SD. Data were derived from six animals in each group. Comparisons were performed using one-way ANOVA or Kruskal-Wallis followed by Fisher's PLSD test. Statistical significance was defined as  $p < 0.05$ .

## Results

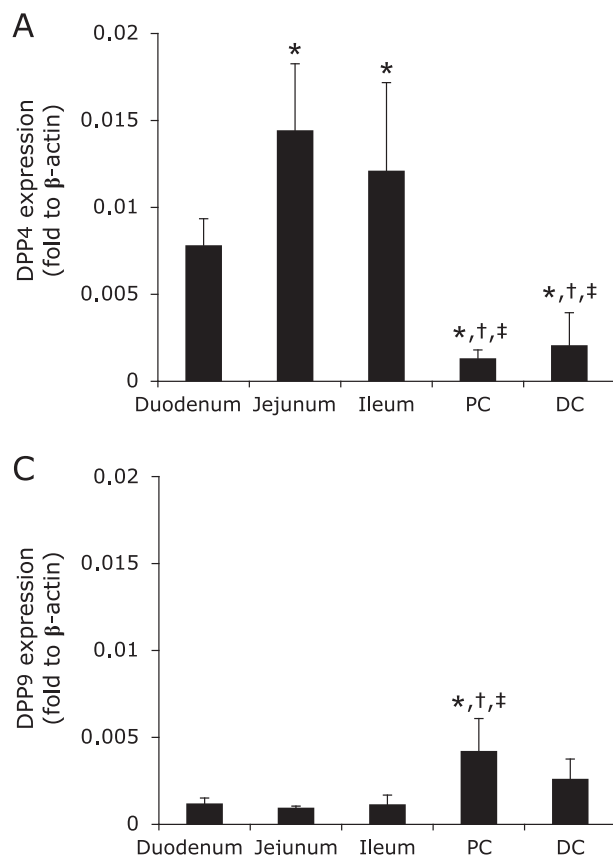
**Mucosal DPP4 mRNA expression and enzyme activity in murine intestine.** We first examined segmental differences in DPP mRNA expression in normal murine intestine. Consistent with previous reports,<sup>(25,26)</sup> real-time PCR analysis revealed the highest DPP4 mRNA expression in the jejunum and ileum, whereas expression was low in the PC and DC (Fig. 1A). Other DPP gene family members, DPP8 and DPP9, were predominantly expressed in the colon, but less so in the duodenum and small intestine (Fig. 1B and C). Another DPP, fibroblast activation protein, was not detected in any intestinal segment tested (data

not shown).

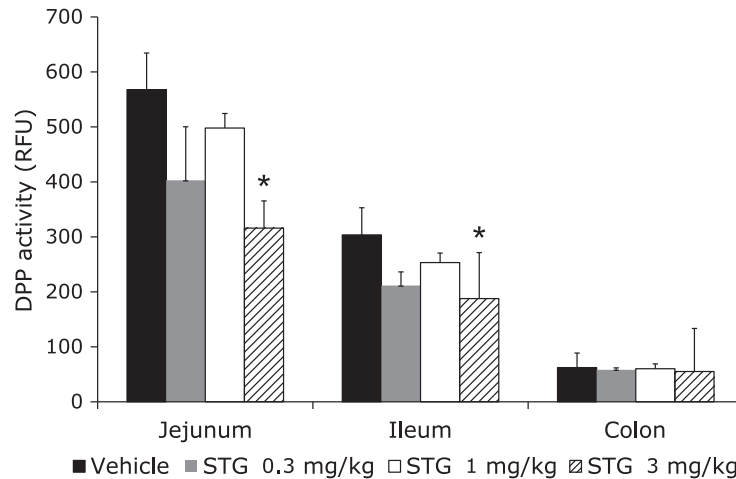
Next, we measured mucosal DPP activity in the duodenum, jejunum, ileum, and colon. Although DPP8, DPP9, and FAP have activity similar to DPP4, the DPP activity assay revealed that the distribution of enzyme activity and DPP4 mRNA expression were highly correlated (Fig. 2), suggesting that DPP activity measured in each segment corresponds to DPP4 expression.



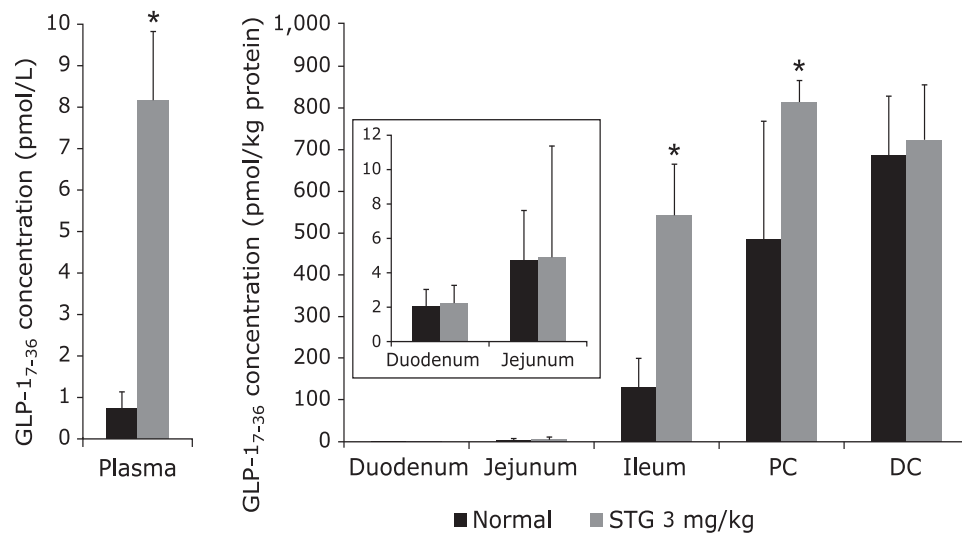
**Fig. 2.** DPP enzymatic activity in the duodenum, jejunum, ileum, PC, and DC. DPP activity in mucosa from each area was expressed as relative fluorescence units (RFU). \* $p < 0.05$  vs duodenum, † $p < 0.05$  vs Jejunum, ‡ $p < 0.05$  vs ileum.



**Fig. 1.** Mucosal DPP mRNA expression in the duodenum, jejunum, ileum, proximal colon (PC), and distal colon (DC). mRNA expression of DPP4 (A), DPP8 (B), and DPP9 (C) was measured and expressed relative to β-actin. Values were expressed as means ± SD. \* $p < 0.05$  vs duodenum, † $p < 0.05$  vs jejunum, ‡ $p < 0.05$  vs ileum.



**Fig. 3.** Effects of sitagliptin (STG) on mucosal DPP enzyme activity in the duodenum, jejunum, and colon. DPP enzymatic activity was measured in intestinal segments from vehicle- or STG-treated mice (0.3–3 mg/kg, ig). \* $p < 0.05$  vs vehicle.



**Fig. 4.** Effects of sitagliptin (STG) on GLP-1<sub>7-36</sub> concentrations in plasma, and duodenum, jejunum, ileum, and proximal and distal colon in mice. \* $p < 0.05$  vs vehicle.

**Effects of STG on plasma and mucosal DPP4 activity in mouse intestine.** The effects of STG (0.3–3 mg/kg, i.g.) on plasma and mucosal DPP4 activity were measured 24 h after treatment (Fig. 3). Although plasma DPP4 activity (data not shown) and mucosal DPP4 activity were not significantly reduced in mouse colon 24 h after treatment, oral administration of STG dose-dependently suppressed mucosal DPP activity in the jejunum and ileum.

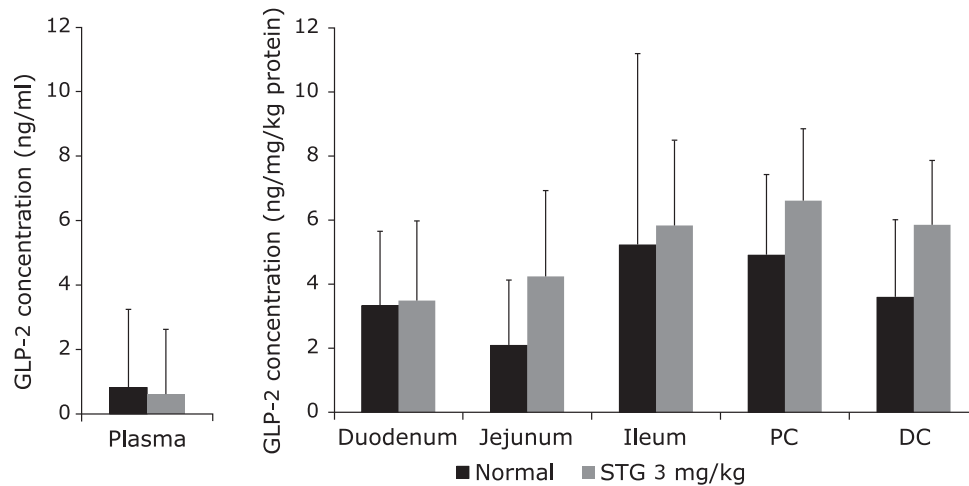
**Effects of STG on plasma and mucosal GLP concentrations in mouse intestine.** Plasma concentrations of GLP-1<sub>7-36</sub> were significantly higher in STG-treated mice than in vehicle-treated mice (Fig. 4). STG also significantly increased mucosal GLP-1<sub>7-36</sub> concentrations in the ileum and PC (Fig. 4). However, STG treatment did not affect plasma or mucosal total GLP-2 concentrations (Fig. 5).

**Preventive effects of STG on IM-induced intestinal ulcers in rats.** Pretreatment with STG (3 mg/kg, i.g., for 2 days on Days –1 and 0) reduced intestinal ulcers compared to the vehicle + IM group (Fig. 6A and B). STG dose-dependently reduced ulcer scores (Fig. 6D). Co-administration of GLP-2<sub>3-33</sub>

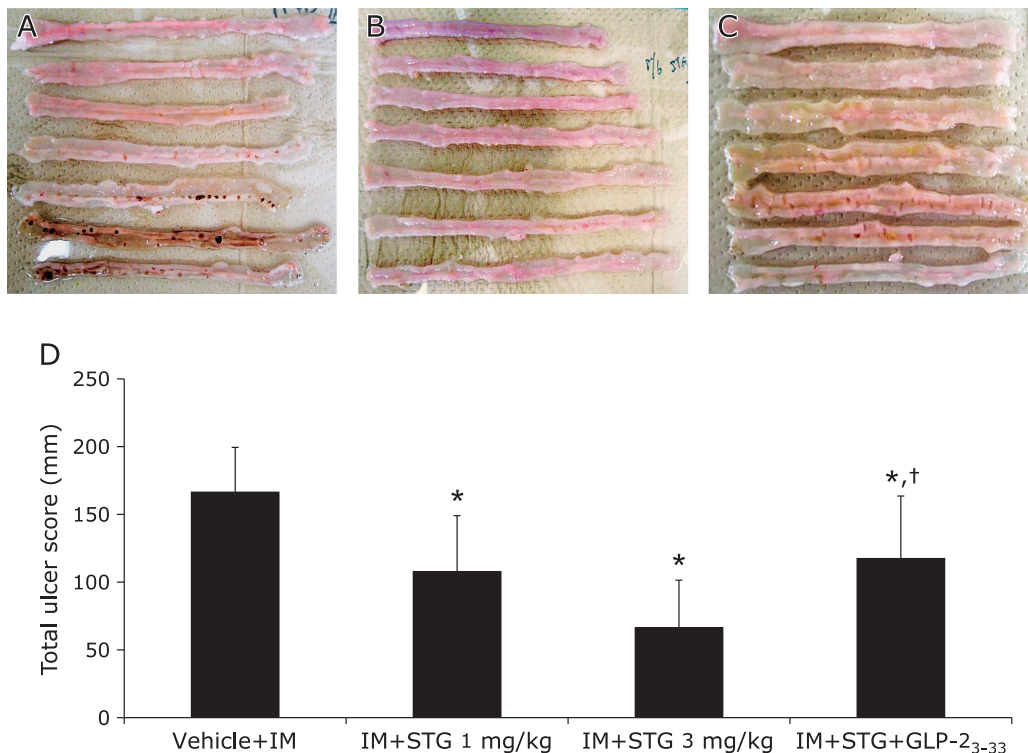
(100 µg/kg/day, s.c., for 2 days on Days –1 and 0) partially reversed the preventive effect of STG on ulcer formation (Fig. 6C and D).

Immunohistochemical examination revealed increased ileal ssDNA staining after IM treatment, which was suppressed by pretreatment with STG (Fig. 7A–C). Furthermore, Western blotting revealed that expression of cleaved caspase-3 was also reduced in the ileum in the STG + IM group (Fig. 7D).

**The healing effect of STG and ED on IM-induced intestinal ulcers.** Seven days after IM treatment, ulcers remained present, mostly in the ileum. Daily treatment with ED after IM treatment significantly reduced the ulcer score compared to the IM + vehicle group (Fig. 8). Although STG alone had no significant healing effect on intestinal ulcers, ED with STG treatment promoted ulcer healing. Co-administration of GLP-2<sub>3-33</sub> (100 µg/kg/day, s.c., Day 2 to 8) partially reversed ED + STG-induced healing of intestinal ulcers. During healing, IM treatment reduced total GLP-2 concentrations in the ileal mucosa (Fig. 9). ED and ED + STG treatment significantly increased mucosal total GLP-2 concentrations, whereas STG alone had no significant effect.



**Fig. 5.** Effects of sitagliptin (STG) on plasma and mucosal concentrations of GLP-2 in mouse intestine.



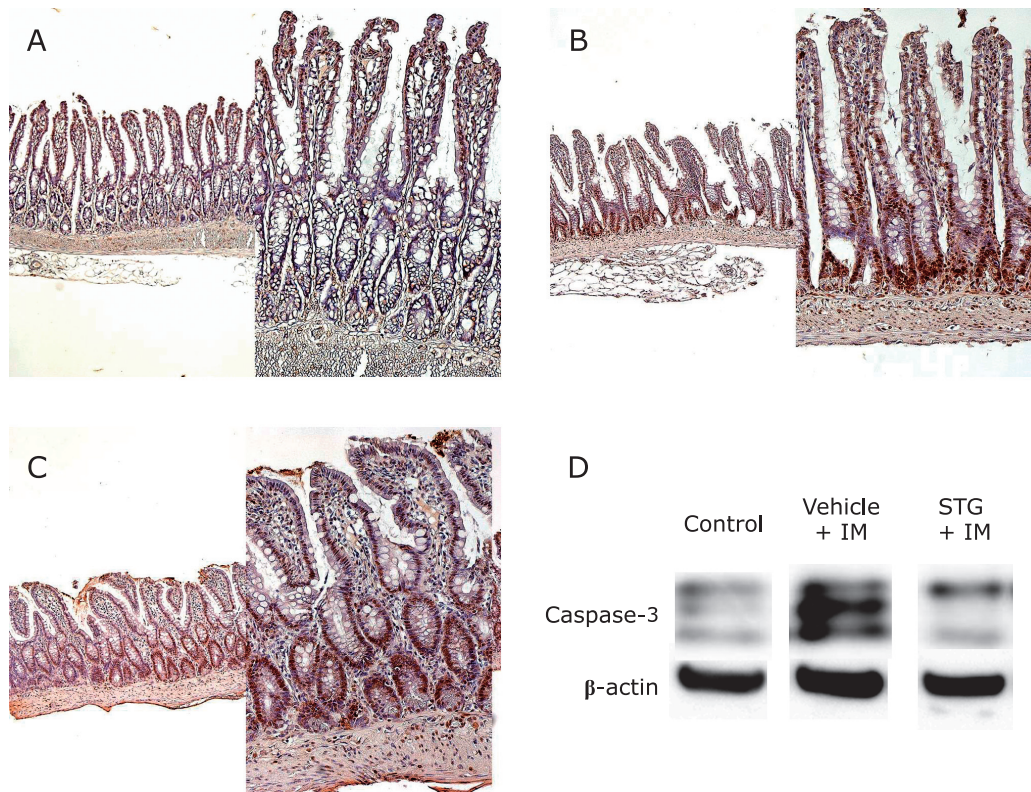
**Fig. 6.** Effects of sitagliptin (STG) on intestinal ulcer formation in rats. The small intestine was divided into 7 segments: 1 duodenal, 2 jejunal, and 4 ileal segments. Intestinal ulcers were induced by indomethacin (IM) treatment (10 mg/kg, s.c.). Representative macroscopic images of small intestinal ulcers are shown; A: Vehicle + IM, B: STG (3 mg/kg, i.g.) + IM, C: STG (3 mg/kg, i.g.) + GLP-2<sub>3-33</sub> (100 µg/kg, s.c.) + IM. D: Total ulcer scores. STG pre-treatment reduced total ulcer score, whereas GLP-2<sub>3-33</sub> partially reversed the effects of STG. \* $p < 0.05$  vs vehicle + IM group, † $p < 0.05$  vs STG (3 mg/kg, i.g.) + IM group.

## Discussion

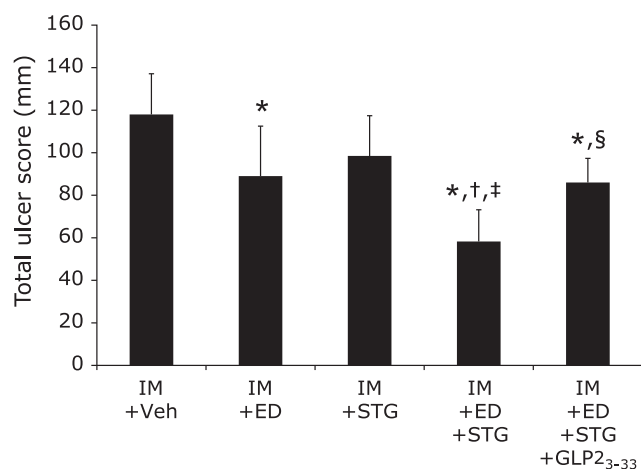
We demonstrated that the DPP4 inhibitor STG suppressed mucosal DPP4 activity and increased mucosal concentrations of GLP-1<sub>7-36</sub> in the ileum and proximal colon, accelerating intestinal ulcer healing. We also report that feeding an elemental diet increased ileal mucosal concentration of total GLP-2. Furthermore, STG administration prevented ulcer formation, and when

combined with ED treatment, accelerated healing of IM-induced intestinal ulcers via a GLP-2 pathway, consistent with our recent study.<sup>(22)</sup> We have previously reported that DPP4 inhibition potentiated the effects of luminal nutrients, such as amino acids, via a GLP-2 pathway, and suggested that the modulation of the local concentration by release of the endogenous hormone GLP-2 by luminal compounds and DPP4 inhibition provide mucosal protection.<sup>(27,28)</sup> Taken together, these findings indicate that DPP4

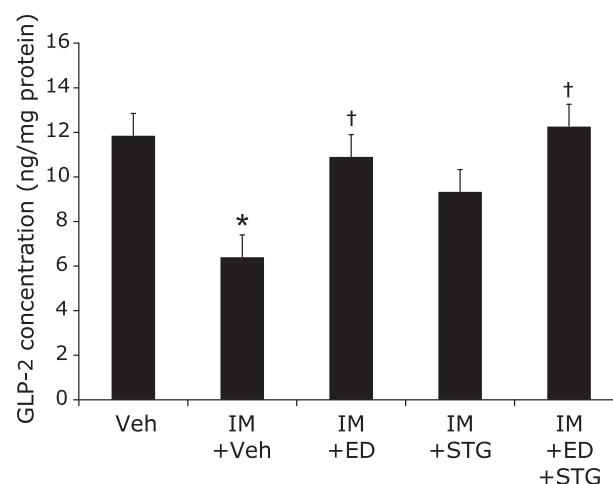




**Fig. 7.** Effects of sitagliptin (STG) on epithelial apoptosis in the ilea of indomethacin (IM)-treated rats. A grossly normal ileum lesion was immuno-stained for single-stranded DNA (A–C; left  $\times 100$ , right  $\times 200$ ) and immunoblotted for cleaved Caspase-3 (D). A: control, B: Vehicle + IM, C: STG (3 mg/kg, i.g.) + IM.



**Fig. 8.** Effects of sitagliptin (STG) on intestinal ulcer healing in rats. Intestinal ulcers induced by indomethacin (IM, 8 mg/kg, s.c., once per day on Days 0 and 1) were assessed on Day 8 with or without daily treatment with vehicle (Veh), an elemental diet (ED) and/or STG (3 mg/kg, i.g.) from Day 2 to Day 8. ED + STG treatment significantly reduced total ulcer score on Day 8. Co-administration of GLP-2<sub>3-33</sub> (100  $\mu$ g/kg/day, sc, for 7 days) partially reversed the healing effect of ED + STG on the intestinal ulcers. \* $p < 0.05$  vs IM + Veh, † $p < 0.05$  vs IM + ED, ‡ $p < 0.05$  vs IM + STG, § $p < 0.05$  vs IM + ED + STG.



**Fig. 9.** Mucosal concentration of total GLP-2 in the ilea of indomethacin (IM)-treated rats during ulcer healing. IM treatment on Days 0 and 1 reduced total GLP-2 concentration in the ileum on Day 8. An elemental diet (ED) and ED + sitagliptin (STG) treatment significantly reversed the reduced concentration of total GLP-2. \* $p < 0.05$  vs vehicle (Veh), † $p < 0.05$  vs IM + Veh.

inhibition combined with luminal nutrients may be an attractive candidate for regulating intestinal mucosal GLP concentrations, and may be therapeutically useful for the treatment of NSAID-induced intestinal injury.

DPP4 is a member of the serine protease family, which also includes DPP8 and DPP9.<sup>(10)</sup> DPP4 cleaves a number of regulatory factors including hormones and cytokines.<sup>(29)</sup> Since the insulino-tropic hormone GLP-1 is rapidly degraded and deactivated by

DPP4, DPP4 inhibitors have recently emerged as a treatment for type 2 diabetes. These orally active drugs increase active serum GLP-1 concentrations, which in turn increase insulin secretion and improve glycemic control.<sup>(30)</sup> GLP-2, which like GLP-1 is derived from proglucagon in enteroendocrine L cells, enhances intestinal ion secretion and cell growth, and is clinically useful for the treatment of short bowel syndrome and Crohn's disease.<sup>(31,32)</sup> Since GLP-2 is also rapidly degraded and deactivated by DPP4, inhibition of DPP4 has been considered a novel approach for the treatment of inflammatory bowel disease.<sup>(10)</sup> Our study revealed high levels of expression of DPP4 mRNA in jejunal and ileal mucosa, but not in colonic mucosa.

Although higher levels of expression of DPP8 and DPP9 mRNA were detected in the colon than in the small intestine, DPP activity was much higher in the small intestine than in the colon. Moreover, the DPP4 inhibitor STG significantly suppressed mucosal DPP activity in the jejunum and ileum, but not in the colon. These results suggest that DPP enzymatic activity in the colon is mainly a result of DPP8 and DPP9 activity, whereas DPP activity in the small intestine corresponds to DPP4 activity. Therefore, DPP4 inhibitors likely selectively enhance local jejunal and ileal GLP-2 concentrations but may have little effect on colonic local GLP-2 concentrations. Similar results of mRNA and enzyme activities were observed in rat intestine (data not shown). Similar results of mRNA and enzyme activities were observed in rat intestine (data not shown). Yu *et al.*<sup>(33)</sup> reported high expression of DPP8 and DPP9 in the colon that was upregulated in DPP4 knockout mice. Since compensatory upregulation implies overlapping enzyme function, it is likely that the DPP family members are functionally similar.<sup>(33)</sup> Using an experimental colitis model in mice, Geier *et al.*<sup>(13)</sup> and Yazbeck *et al.*<sup>(34)</sup> also found that DPP4 knockout mice still exhibit significant DPP activity, again likely due to compensatory upregulation. Therefore, DPP4 inhibition may not be suitable for treatment of inflammatory diseases of the colon, but useful for ileal diseases, where DPP4 is predominant.

Mucosal DPP4 activity was significantly decreased by oral administration of STG, which significantly elevated the ileal mucosal concentration of GLP-1<sub>7-36</sub>. STG treatment prevented intestinal ulcer formation and potentiated ED-induced promotion of ulcer healing via the ileal GLP-2 pathway, suggesting that STG may increase mucosal GLP-2 concentrations. These results

suggest that STG may be clinically useful for the treatment of ileal diseases, such as Crohn's disease and NSAID-induced enteritis. STG treatment did not alter mucosal GLP-2 concentrations, presumably because only total GLP-2 was measured. The measurement of bioactive GLP-2 will clarify whether DPP4 inhibition increases mucosal active GLP-2 concentrations as it does for active GLP-1.

Interestingly, plasma concentrations of GLP-1<sub>7-36</sub> were also elevated, even though STG administration reduced mucosal DPP activity, but did not significantly suppress plasma DPP activity. Therefore, intestinal mucosal DPP4 activity rather than plasma DPP activity may primarily regulate plasma GLP concentration. Inhibition of mucosal DPP4 activity may potentiate the local effects of GLP-2 in small intestine.

In conclusion, mucosal DPP4 inhibition may effectively treat NSAID-induced small intestinal ulcers via activation of the GLP-2 pathway. ED treatment promoted intestinal ulcer healing and augmented mucosal GLP-2 concentrations, enhanced by DPP4 inhibition, suggesting that the combination of DPP4 inhibition with luminal amino acids may be therapeutic for ileal diseases, such as intestinal Crohn's disease and NSAID-induced enteropathy.

## Abbreviations

DC	distal colon
DPP4	dipeptidyl peptidase 4
ED	elemental diet
GIP	gastric inhibitory polypeptide/glucose-dependent insulinotropic peptide
GLP	glucagon-like peptide
IM	indomethacin
NSAIDs	non-steroidal anti-inflammatory drugs
PC	proximal colon
ssDNA	single-stranded DNA
STG	sitagliptin
TBST	Tris-buffered saline with Tween

## Conflict of Interest

No potential conflicts of interest were disclosed.

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